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THE PEEL METHOD IN PALEOBOTANY

BY

WILLIAM C. DARRAH

THE peel method of making microscopic preparations has become popular in many paleontological laboratories since its development ten years ago. The possibilities of diverse applications of the method have not been realized, because it has been primarily used in the study of coal-balls. Coal-balls are fossil-bearing dolomitic nodules found in association with a small number of coal beds. Professor Walton[1] devised a method of making serial sections for English coal-balls. Professor Leclercq[2] has been using the Walton method with excellent results on Belgian coal-balls. Doctor Roy Graham[3] made certain modifications in order to adapt it to Illinois coal-balls. Other investigators[4] have used Graham's procedure for calcified animal remains.

By this method successive films of nitrocellulose are peeled from a smoothed surface of a petrifaction. The fossil must first be attacked by an acid in order to remove the rock matrix and cause the carbonized cell walls to stand out in relief. Then a solution similar to collodion is poured over the "etched" surface and permitted to dry. The dried film is carefully peeled off. The procedure may be repeated many times.

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[1] C. R. Congress Adv. Strat. Carb. Heerlen, pp. 749-754.

[2] Ann. Soc. Geol. Belg., vol. 52.

[3] Stain Technology, vol. 8:65-68.

[4] Am. Midl. Nat., vol. 16:410-412.

In addition to this method of making multiple peels from one specimen, paleobotanists, since the time of Nathorst, have "transferred" various types of carbonizations and incrustations. Walton[5] has fully described this method. The thin, but nevertheless three-dimensional fossil is cemented to a glass slide with collodion or balsam. Then the glass slide is covered completely with paraffin. Finally the specimen is placed in a container of hydrofluoric or nitric acid and allowed to remain in the acid until all mineral has been removed. Obviously this process is very slow.

For the past five years various experiments have been made with the result that these two types of work can be done with one simple, time-saving technique. Improved solutions have been developed to aid in the preparation of satisfactory peels.

The possibilities of microtechnique in paleobotany have not been recognized, or even admitted by many botanists and geologists. Nevertheless, the trained paleobotanist can obtain through microtechnique the maximum available information from any specimen with preserved structure. Even the simple carbonized impression, long considered worthless as a source of biological data, may be studied without difficulty by the peel method.

The complete process of making a nitrocellulose peel involves a number of steps. These are in order: grinding, etching, drying, smearing, peeling, and care of peels.

Grinding. Any petrified specimen in which chemical replacement has occurred may be studied by the preparation of multiple peels (or serial-sections). It is necessary to grind a series of smooth surfaces at pre-determined intervals. It is possible to make from 250 to 500 peels per inch although such frequency is rarely desirable. The actual amount of fossil in the peel is from one to three micra in thickness. In order to guarantee uniform intervals between peels, the specimen must be calibrated. Small or fragile specimens should first be imbedded in plaster or Wood's metal. The latter is excellent but, unfortunately, expensive since several pounds are needed to imbed even a small specimen. The actual grinding is done with a fine or medium carborundum powder. Number 320 will do, 600 is better, and any size intermediate will give good results. Although grinding may be done by hand, a revolving lap is highly recommended for exact work. The abrasive powder is placed upon the lap and then moistened with water. The lap is set in motion (or the specimen is rotated by hand in one direction only) and the surface is smoothed and ground to the desired distance. The lap must be continuously moistened. If abrasive powders of different fineness are used, coarse should not be permitted to contaminate fine, otherwise deep scratches will mar both the smoothed surface and the lap surface.

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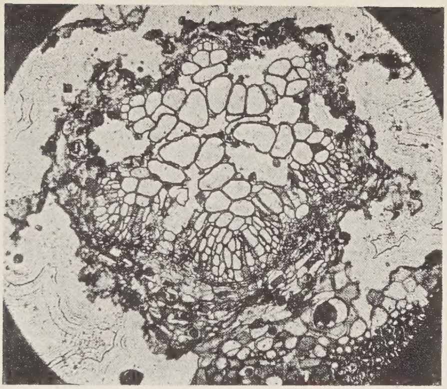
[5] loc. cit.

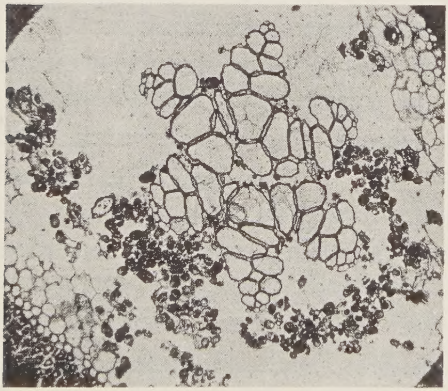
Etching. Etching is the process by which the rock matrix is removed, with acid, from the carbonized structure. It is this process which may lead to success or failure, because careless etching destroys the cellular detail and frays the outline of the cell walls. It is necessary to use not only different kinds of acids but also different concentrations. Ordinary calcified specimens may be etched with a 1% solution of hydrochloric acid for 30 seconds. Dolomitic specimens which contain magnesium carbonate may require a 10% solution for one minute.

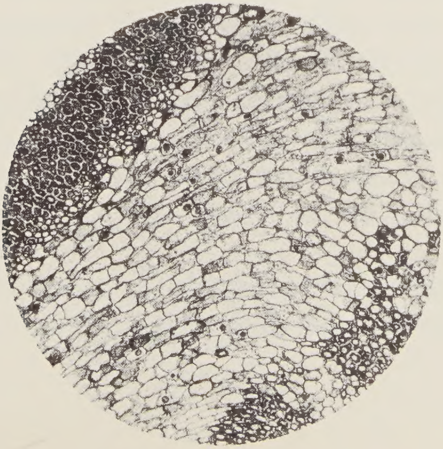
Silicified specimens must be etched with commercial hydrofluoric acid (49%) for three minutes, although sub-

EXPLANATION OF THE ILLUSTRATIONS

Psaronius *sp. nov.* Heliotype reproductions of photographs of nitrocellulose peels from a silicified stem. Figure at top shows the stele of a cortical root with some secondary activity. Middle figure shows a typical root with well preserved cell-walls. Lower figure shows sclerenchymatous tissue between roots. Magnified 44 times. Carboniferous: Illinois.







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sequent peels may be made with a 25% solution acting for two minutes. The first etching frequently makes the specimen more susceptible to subsequent attacks.

Partially silicified specimens may be etched the first time with 25% hydrofluoric acid and thereafter by 10% hydrochloric acid for two minutes.

Hydrofluoric acid must be used with great caution because of its dangerous corrosive action, especially on the mucous membranes and on the fingernails.

Coals require special softening treatment before they can be etched. This will be discussed later.

Etching is a trial and error process. All kinds of petrifaction occur and frequent trials may be necessary before a suitable concentration is secured. Under ordinary circumstances the etched surface is carefully washed with running water in order to remove excess acid.

Drying. The etched specimen must be air-dried in a dust-free place. If one is making a number of peels, it is frequently helpful on a humid day, to set in motion an air current by means of a small electric fan. After the specimen has thoroughly dried, it is placed in a level position on plastilene bases or in a sand-box.

Smearing. The etched surface must be carefully covered with a nitrocellulose solution. The solution can be applied by two means: it may be poured gradually over the surface by a continuous slow flow from a bottle, or it may be smeared by drawing a glass stirring rod over the specimen. We pour the solution, because fewer air bubbles form on the surface. Bubbles may be punctured with a dissecting needle or removed with a scalpel. If, upon drying, the film is too thin to allow removal, additional solution may be poured on the surface and then permitted to dry.

Various kinds of solutions have been used by individual investigators. The following formula will give good results on any type of specimen. It gives a durable, pliable, transparent film which will not "yellow" or harden with age. The consistency is viscous enough to minimize the occurrence of air bubbles.

Parlodion (Mallinckrodt) . . .	28 grams
Butyl Acetate (commercial) . .	250 cc.
Amyl Alcohol	30 cc.
Xylol (or Toluene)	10 cc.
Castor Oil	3 cc.
Ether	3 cc.

The solution should be allowed to stand a week before being used.

Parlodion is rather expensive, but the results are much superior to those obtained by guncottons (commercial nitrocellulose). We have compared a large series of nitrocellulose substances in our experiments. The majority showed a definite tendency to "yellow" and "brittle."

Chloroform may be substituted for ether. This is added to hasten hardening along the edges of the film. Castor oil makes the film pliable. Amyl acetate can be used as a substitute for butyl acetate, and in this case no amyl alcohol is needed.

Peeling. The solution dries slowly and will not be ready for removal for six or eight hours. Peels with a surface greater than 9 square inches will not dry in less than 12 hours. The surface of the specimen is "peeled" with the film, by first loosening one edge, and then carefully pulling off the whole film. We have prepared peels up to the size of eighty square inches, and such peels possess a uniform thickness and toughness. A thoroughly dried film will not wrinkle or curl after removal.

Care of Peels. Some workers prefer to file the peels in envelopes or folders. No special care is really necessary. However, one cannot subject an unmounted peel to high magnification, and its great usefulness is not realized. With a low-power binocular microscope, one can select desirable portions of a peel for permanent mounting. Such portions are cut out and trimmed with a sharp scissors and are mounted in balsam-"damar" (in xylol) on a glass slide of good quality (specifically, "non-corrosive"). The peel should be covered with a number one cover-slip and weighted with a small lead block until absolutely dry. These permanent mounts are frequently capable of being magnified from 1000 to 1200 times, and 440 is average.

Occasionally so little carbon is preserved, that the peel shows only cell outline. Such a peel may be stained with any of the common biological or petrological stains, such as Light Green, Saffranin, Erosin, or Nigrosene. One is surprised to learn that it is sufficient to immerse the peel in a 2% aqueous solution of stain, and then wash out the excess dye.

The peel has tremendous advantages over the ground-section because it is thinner, more translucent, less costly and quicker to make. The peel from an incrustation or carbonization is a new departure in paleobotany and has not been reported previously.

The methods thus far described are primarily adapted to calcareous, dolomitic, and silicified specimens. We have had success with the following materials, and thus far have not experienced any failures:

Coal-balls = England, Belgium, Holland, United States.
Calcareous petrifactions = Pennsylvania, New York, England, France.
Silicified petrifactions = Rhynie (Scotland), Grand Croix (France), Psaronius from Ohio, Saxony, Illinois.
Coniferous woods from New Mexico, England,

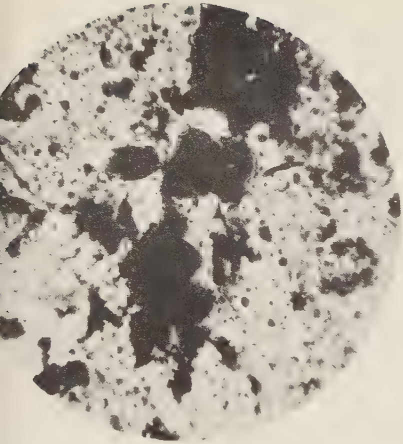
EXPLANATION OF THE ILLUSTRATIONS

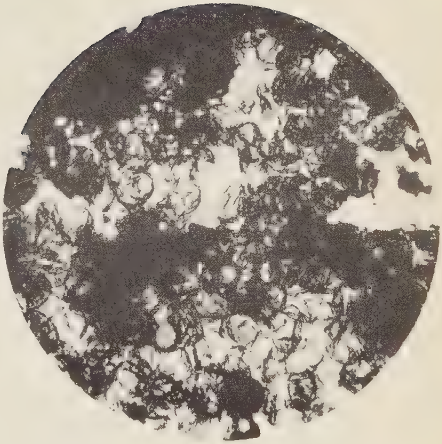
Figure at upper left. CODONOTHECA CADUCA *Sellards.* The cluster of carbonized spores has been peeled from an incrustation. Magnified 44 times. Carboniferous: Mazon Creek, Illinois. Thompson Collection.

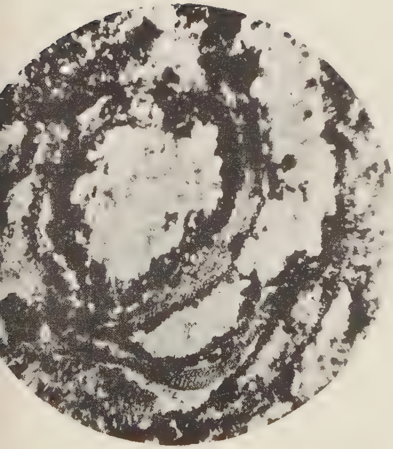
Figure at upper right. CROSSOTHECA SAGITTATA (*Lesquereux*) *Sellards.* This peel shows a mass of spores from a carbonization. Magnified 60 times. Carboniferous: Mazon Creek, Illinois. Thompson Collection.

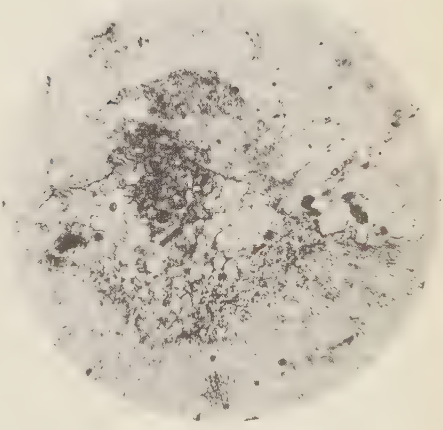
Figure at lower left. CORDAIANTHUS sp. The peel shows the longitudinal section through a female "flower" with sterile bracts at the base. Magnified 24 times. Carboniferous: Mazon Creek, Illinois. Thompson Collection.

Figure at lower right. PALEOMYCES sp. This peel taken from a necrotic area in a silicified stem in *Rhynia major* Kidston and Lang shows the diseased tissue and several small spores around the periphery in the "gelatinous" sheath. Magnified 60 times. Devonian: Rhynie, Scotland.









Ohio, Germany.

Nematophyton from Canada.

Carbonized impressions = Mazon Creek (Illinois), Pennsylvania, Holland, England, France, Germany, Switzerland.

Bituminous coal = Pennsylvania, Ohio, Antarctic.

Anthracite coals = Pennsylvania.

Peeling the Carbonization. This procedure is based upon the schedules used for four types of specimens: a Carboniferous calamite cone (*Macrostachya Thompsonii* Darrah) with spores, sporangia, and sporangiophores, all preserved; a Carboniferous fern fructification (*Crossotheca sagittata* (Lesquereux) Sellards) showing spores in place; a Triassic cycadean frond from which was obtained cuticles with the stomata preserved; and a Miocene leaf with basidiomycete decay spots. Before the specimen is etched, it is necessary to lay bare the sporangia with a fine chisel and a jeweler's hammer.

On a carbonization or incrustation the surface to be etched is *more or less* even. The acid solution (usually 1% to 5% hydrochloric) is carefully applied and the excess removed with water. After the specimen dries, nitrocellulose solution is poured over the surface. If the specimen is very uneven, it may be desirable to build up the peel by several layers of solution. The dried peel, after removal, should be flattened under a weight in a warm-chamber of 140° F. for an hour.

There is scarcely a limit to which this peel method may be applied in paleobotany—provided that cellular structure is preserved and that the rock can be etched or attacked with an acid. Graphitized impressions can be removed from slate, but the cell structure has been destroyed during metamorphism.

Coal. We have had some success with coal. Although improvements must and will be made, it is of use to indicate what can be done in the study of higher rank coals. Coal by nature is a flattened mass of carbonized plant debris with a comparatively small amount of mineral in the form of ash. Coal may be studied microscopically by four methods: the ground section with transmitted light; the polished surface by reflected light (Duparque, Koopmans); the imbedded, softened, demineralized, microtome section (Jeffrey[6]); and now the nitrocellulose peel. The most superior of these is the microtome method, especially good for lignitic, bituminous, and cannel coals, but not yet adapted to anthracites. Neither the polished nor the ground section can be greatly magnified. The peel method is the only fairly quick method available, and the only method applicable to the study of anthracites.

The coal specimen must be ground smooth on the surface to be peeled. This surface must be demineralized (on the surface only) by Schultze's macerating fluid at a temperature of 180° F. This surface is washed with water and covered with a 20% solution of potassium hydroxide in alcohol. After washing again it is bathed with a 25% solution of phenol and chromic acid. With anthracite coal a 25% solution of hydrofluoric acid is used instead of chromic acid. After a thorough washing with water, the coal is dried and smeared with nitrocellulose solution.

Somewhat better peels have been made on anthracites than on bituminous coals.

It is also well to record two difficulties encountered in the nitrocellulose techniques. One is shrinkage of the peel which takes place during drying, and which is caused by the evaporation of volatile materials in the solution. This shrinkage is partially overcome by slow drying. It is also lessened by leaving the dry peel on the specimen as long as possible—even two days is not too long. The shrinkage of a peel 50 millimeters long is approximately 2 millimeters if drying has taken less than 12 hours. The other difficulty has been indicated before, that is the development of air-bubbles. This is chiefly overcome by mixing a slow-drying solution with Parlodion, such as that recommended, and by thoroughly drying the specimen before applying the nitrocellulose solution. It is sometimes helpful to moisten the surface with butyl acetate. Collodion and similar substances invariably develop many small bubbles, because of their rapid drying nature.

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[6] Sci. Conspectus, vol. 6: 71-76.

In addition to these purely paleobotanical uses of the peel technique, there are many interesting applications in other fields of work. We have tried similar methods on recent and fossil animals with calcareous parts and on anthropological material such as carbonized grains and sun-dried bricks. Some petrographic observations can be readily made with the peel technique.

To my wife, Helen Hilsman Darrah, I wish to extend thanks and to acknowledge her part in the development of various phases of this nitrocellulose peel technique.

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